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 Petersen, Steen Guldager
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<120> IMPROVED METHOD OF PRODUCING AN ASPARTIC PROTEASE POLYPEPTIDE IN A RECOMBINANT HOST ORGANISM

<130> P1031US00

<150> PA 2002 0092

<151> 2002-06-17

<160> 10

<170> PatentIn version 3.3

<210> 1

<211> 323

<212> PRT

<213> Bos taurus

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Phe Gly Lys Ile Tyr Leu Gly Thr Pro Pro Gln Glu Phe Thr Val Leu 20 25 30

Phe Asp Thr Gly Ser Ser Asp Phe Trp Val Pro Ser Ile Tyr Cys Lys 35 40 45

Ser Asn Ala Cys Lys Asn His Gln Arg Phe Asp Pro Arg Lys Ser Ser 50 55 60

Thr Phe Gln Asn Leu Gly Lys Pro Leu Ser Ile His Tyr Gly Thr Gly 65 70 75 80

Ser Met Gln Gly Ile Leu Gly Tyr Asp Thr Val Thr Val Ser Asn Ile 85 90 95

Val Asp Ile Gln Gln Thr Val Gly Leu Ser Thr Gln Glu Pro Gly Asp 100 105 110

Val Phe Thr Tyr Ala Glu Phe Asp Gly Ile Leu Gly Met Ala Tyr Pro 115 120 125 Ser Leu Ala Ser Glu Tyr Ser Ile Pro Val Phe Asp Asn Met Met Asn 130 135 140

Arg His Leu Val Ala Gln Asp Leu Phe Ser Val Tyr Met Asp Arg Asn 145 150 155 160

Gly Gln Glu Ser Met Leu Thr Leu Gly Ala Ile Asp Pro Ser Tyr Tyr 165 170 175

Thr Gly Ser Leu His Trp Val Pro Val Thr Val Gln Gln Tyr Trp Gln
180 185 190

Phe Thr Val Asp Ser Val Thr Ile Ser Gly Val Val Val Ala Cys Glu 195 200 205

Gly Gly Cys Gln Ala Ile Leu Asp Thr Gly Thr Ser Lys Leu Val Gly 210 215 220

Pro Ser Ser Asp Ile Leu Asn Ile Gln Gln Ala Ile Gly Ala Thr Gln 225 230 235 240

Asn Gln Tyr Gly Glu Phe Asp Ile Asp Cys Asp Asn Leu Ser Tyr Met 245 250 255

Pro Thr Val Val Phe Glu Ile Asn Gly Lys Met Tyr Pro Leu Thr Pro 260 265 270

Ser Ala Tyr Thr Ser Gln Asp Gln Gly Phe Cys Thr Ser Gly Phe Gln 275 280 285

Ser Glu Asn His Ser Gln Lys Trp Ile Leu Gly Asp Val Phe Ile Arg 290 295 300

Glu Tyr Tyr Ser Val Phe Asp Arg Ala Asn Asn Leu Val Gly Leu Ala 305 310 315 320

Lys Ala Ile

<210> 2

<211> 1142

<212> DNA

## <213> artificial

<220>

<223> DNA fragment comprising a DNA fragment of 1138 bp designed to comprise a N-H-T glycosylation site and unique SalI and XhoI sites for cloning purposes (modB-XS).

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aagggcaagt	ctctgcgtaa	ggctctcaag	gagcacggtc	tgctcgagga	tttcctgcag	120
aagcagcagt	acggcatcag	ctctaagtac	agcggtttcg	gcgaggtggc	cagcgtgcct	180
ctcactaact	acctggacag	ccagtacttc	ggtaagatct	accttggcac	tcccctcag	240
gagttcaccg	ttctgttcga	tactggttcc	agcgacttct	gggttccctc	catctactgt	300
aagagcaacg	cttgcaagaa	ccaccagcgc	ttcgatcctc	gcaagtccag	caccttccag	360
aaccttggca	agcccctttc	catccactac	ggtactggca	gcatgcaggg	tatccttggc	420
tacgacaccg	ttaccgtgtc	caacatcgtc	gatattcagc	agaccgtggg	tctgagcacc	480
caggagcctg	gcgatgtctt	cacttacgcc	gagttcgatg	gtatcctcgg	catggcttac	540
ccctccctgg	cctctgagta	ctctatccct	gtgttcgaca	acatgatgaa	ccgccacctc	600
gtcgctcagg	atctgttcag	cgtgtacatg	gaccgtaacg	gtcaggagtc	catgcttact	660
ctgggcgcca	tcgatccctc	ttactacacc	ggttccctcc	actgggttcc	tgtgaccgtc	720
cagcagtact	ggcagttcac	cgtggacagc	gtcactatct	ccggcgtggt	tgtggcttgc	780
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gacatcctga	acatccagca	ggctatcggt	gccacccaga	accagtacgg	cgagttcgat	900
atcgactgcg	ataacctttc	ttacatgcct	actgtggttt	tcgagatcaa	cggtaagatg	960
taccccctta	ctccttctgc	ttacacttcc	caggatcagg	gcttctgtac	ctctggtttc	1020
cagtctgaga	accacagcca	gaagtggatc	cttggcgatg	tcttcatccg	cgagtactac	1080
tccgtcttcg	accgtgccaa	caacctggtg	ggtctcgcta	aggccatctg	atcctctaga	1140
gt						1142

<sup>&</sup>lt;210> 3

<sup>&</sup>lt;211> 408

<sup>&</sup>lt;212> DNA

<sup>&</sup>lt;213> artificial

<sup>&</sup>lt;220>

<sup>&</sup>lt;223> an approximately 410 bp SalI-SphII I fragment made using
 synthetic oligonucleotides (SEQ ID XXX-1)

<400> cggtcga	3 accg	ctacggtgac	tgacacctgg	cgtgccgaga	tcactcgcat	cccctctac	60
aagggca	agt	ctctgcgtaa	ggctctcaag	gagcacggtc	tgctcgagga	tttcctgcag	120
aagcago	cagt	acggcatcag	ctctaagtac	agcggtttcg	gcgaggtggc	cagcgtgcct	180
ctcacta	act	acctggacag	ccagtacttc	ggtaagatct	accttggcac	tececeteag	240
gagttca	accg	ttctgttcga	tactggttcc	agcgacttct	gggttccctc	catctactgt	300
aagagca	aacg	cttgcaagaa	ccaccagcgc	ttcgatcctc	gcaagtccag	caccttccag	360
aacctto	ggca	agcccctttc	catccactac	ggtactggca	gcatgcag		408
<210> <211> <212> <213> <220> <223>		ficial	ly 220 bp Sp	phI-BsrGI fi	ragment made	e using synth	etic
	olig	gonucleotide	es (SEQ ID 1	XXX-2)			
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agcaga	ccgt	gggtctgagc	acccaggagc	ctggcgatgt	cttcacttac	gccgagttcg	120
atggtat	cct	cggcatggct	tacccctccc	tggcctctga	gtactctatc	cctgtgttcg	180
acaacat	gat	gaaccgccac	ctcgtcgctc	aggatctgtt	cagcgtgtac	atg	233
<210><211><211><212><213>	5 200 DNA Arti	ificial					
<220> <223>			ly 190 bp Ba		ragment made	e using synth	etic
<400> gcgtgta	5 acat	ggaccgtaac	ggtcaggagt	ccatgcttac	tctgggcgcc	atcgatccct	60
cttacta	acac	cggttccctc	cactgggttc	ctgtgaccgt	ccagcagtac	tggcagttca	120
ccgtgga	acag	cgtcactatc	tccggcgtgg	ttgtggcttg	cgagggtggc	tgtcaggcca	180
tccttga	atac	tggtaccagc					200

<210> 6 <211> 334

•.		
	<pre> &lt;212&gt; DNA &lt;213&gt; artificial</pre>	
	<220> <223> an approximately 320 bp KpnI-XbaI fragment made using syntheti oligonucleotides (SEQ ID XXX-4)	С
	<400> 6 ctggtaccag caagetegte ggeeeeteea gegacateet gaacateeag caggetateg	60
	gtgccaccca gaaccagtac ggcgagttcg atatcgactg cgataacctt tcttacatgc	120
	ctactgtggt tttcgagatc aacggtaaga tgtaccccct tactccttct gcttacactt	180
	cccaggatca gggcttctgt acctctggtt tccagtctga gaaccacagc cagaagtgga	240
	teettggega tgtetteate egegagtaet aeteegtett egaeegtgee aacaaeetgg	300
	tgggtctcgc taaggccatc tgatcctcta gagt	334
	<pre>&lt;210&gt; 7 &lt;211&gt; 334 &lt;212&gt; DNA &lt;213&gt; artificial  &lt;220&gt; &lt;223&gt; a modified KpnI-XbaI fragment designed for construction of the modBM gene (SEQ ID XXX-5).</pre>	<b>:</b>
	<400> 7 ctggtaccag caagctcgtc ggcccctcca gcgacatcct gaacatccag caggctatcg	60
	gtgccaccca gaaccagtac ggcgagttcg atatcgactg cgataacctt tcttacatgc	120
	ctactgtggt tttcgagatc aacggtaaga tgtaccccct tactccttct gcttacactt	180
	cccaggatca gggcttctgt acctctggtt tccagtctga gaaccacacc cagaagtgga	240
	teettggega tgtetteate egegagtaet acteegtett egacegtgee aacaacetgg	300
	tgggtctcgc taaggccatc tgatcctcta gagt	334
	<pre>&lt;210&gt; 8 &lt;211&gt; 66 &lt;212&gt; DNA &lt;213&gt; artificial  &lt;220&gt; &lt;223&gt; synthetic polylinker (SalI-SphI-BsrGI-KpnI-XbaI) (SEQ ID XXX-6)</pre>	5)
	<400> 8 ggccaggcgc gccttccatg gaagaatgcg gccgctaaac catcgatggc tcgagttggc	60
	gcgcca	66

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<211> 27	
<212> DNA	
<213> Artificial Sequence	
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<210> 10	
<211> 74	
<212> DNA	
<213> Artificial Sequence	
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<220>	
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4400- 10	
<400> 10	<i>c</i> n
cgtcgaccgc tacggtgact gacacctggc gtaccgacaa ctccaccgag atcactcgca	
tcccctcta caag	74